Characterization of Voltage-Sensitive Ca^{2+} Channels Activated by Presynaptic Glutamate Receptor Stimulation in Hippocampus[a]

C. M. CARVALHO, J. O. MALVA,[b] C. B. DUARTE, AND A. P. CARVALHO

Center for Neurosciences of Coimbra
Department of Zoology
University of Coimbra
3049 Coimbra Codex, Portugal

[b]*Department of Biology*
University of Minho
Braga, Portugal

Glutamate receptor activation modulates the release of several neurotransmitters and neuromodulators. In the hippocampus, the presence of presynaptic glutamate receptors modulating the release of noradrenaline was shown for both NMDA and non-NMDA receptors.[1,2] However, the link between receptor activation and the release of the neurotransmitter has not been clearly established.

In the present study we determined the effects of stimulation by glutamate, AMPA, or NMDA on the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and on the release of [^{3}H]dopamine ([^{3}H]-DA) in hippocampal synaptosomes, and we found that: (1) Ca^{2+} entry due to glutamate receptor stimulation is necessary for [^{3}H]-DA release; (2) AMPA receptor stimulation activates voltage-sensitive Ca^{2+} channels (VSCCs) and a large fraction of Ca^{2+} entry occurs through these VSCCs, because blockade by specific Ca^{2+} channel blockers caused inhibition of [^{3}H]-DA release; (3) the VSCCs involved in the release of [^{3}H]-DA due to AMPA are of the N type (51% inhibition by ω-conotoxin GVIA [ω-CgTx]) and P or Q type (54% inhibition by ω-agatoxin IVA [ω-Aga IVA]); (4) modulation of [^{3}H]-DA release due to NMDA stimulation does not seem to involve activation of VSCCs.

CHANGES IN $[Ca^{2+}]_i$ AND [^{3}H]-DA RELEASE DUE TO AMPA OR NMDA STIMULATION

The results reported in FIGURE 1[3,4] show that stimulation of hippocampal synaptosomes with AMPA (100 μM) increased the $[Ca^{2+}]_i$ by 22.4 ± 1.1 nM and that this effect was inhibited by about 59% in the presence of 10 μM CNQX. NMDA (200 μM) increased the $[Ca^{2+}]_i$ by about 10 nM (FIG. 1A). These changes in $[Ca^{2+}]_i$ are coupled to the Ca^{2+}-dependent [^{3}H]-DA release. Thus, AMPA (100 μM) caused the release of 2.1 ± 0.1% of the total [^{3}H]-DA accumulated, and this effect was reduced to about 60% by 10 μM CNQX (FIG. 1B). NMDA (200 μM) caused the release of

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3.6 ± 0.2% of the total [^{3}H]-DA, and this effect was reduced by 81% by MK-801 (FIG. 1B). Thus, our results show that an apparent exocytotic [^{3}H]-DA release can be triggered by stimulating AMPA or NMDA receptors in hippocampal nerve terminals.

INVOLVEMENT OF VSCCs ON AMPA BUT NOT ON NMDA RECEPTOR STIMULATION

Our results further show that Ca^{2+} entry due to AMPA stimulation occurs mainly through VSCCs, because both the $[Ca^{2+}]_i$ increase and the coupled [^{3}H]-DA release were inhibited by ω-CgTx and ω-Aga IVA (TABLE 1).[3,4] Thus, both ω-CgTx (0.5 μM) and ω-Aga IVA (100 nM) inhibited the change in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) induced by AMPA to 64.8 ± 9.0% and 77.5 ± 4.1% of control, respectively, and this effect was accompanied by an inhibition in [^{3}H]-DA release to 49 ± 3.8% or 46.1 ± 10.5% of control, respectively. No significant inhibitory effects of Ca^{2+} channel blockers were observed on the release of [^{3}H]-DA evoked by NMDA (200 μM), presumably in this case because Ca^{2+} enters mostly through NMDA receptor channels (TABLE 1). Thus,

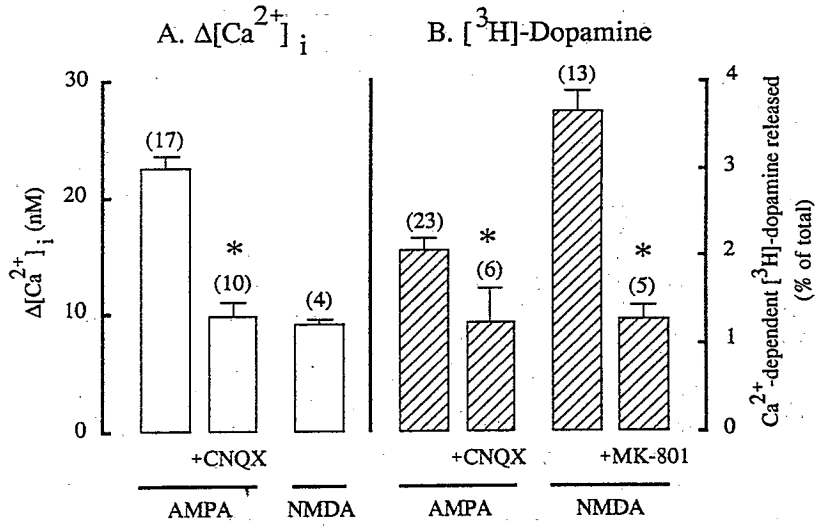

FIGURE 1. Effects of AMPA (100 μM) or NMDA (200 μM) on changes in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) (**A**) and on the release of [^{3}H]dopamine ([^{3}H-DA]) (**B**) in hippocampal synaptosomes. The $[Ca^{2+}]_i$ was determined by a fluorimetric assay using Indo-1 as a probe for Ca^{2+}, and the release of preaccumulated [^{3}H]-DA was studied by using a superfusion system, as described before.[4] Stimulation with AMPA was performed in normal Na^+ medium, whereas NMDA was tested in Mg^{2+}-free Na^+ medium in the presence of glycine (5 μM).[4] The antagonists CNQX (10 μM) and MK-801 (1 μM) were introduced 3 min before stimulation. The [^{3}H]-DA released over basal by AMPA or NMDA, applied for 5 min, was determined in percentage of total [^{3}H]-DA accumulated. High-performance liquid chromatography analysis showed that the released tritium was mainly [^{3}H]-DA (75%), but some [^{3}H]noradrenaline was also present (25%).[4] Data are presented as the mean value ± SEM of the number of ex periments indicated over the bars, performed in different synaptosomal preparations. *Significantly different from the respective control ($p < 0.05$), as determined by the Student t test.

TABLE 1. Relation of $[Ca^{2+}]_i$ Changes to [^{3}H]-Dopamine Release for Various Stimulating Agents and the Effect of Ca^{2+} Channel Blockers in Hippocampal Synaptosomes[a]

Stimulating Agent	Control	Percentage of Control		
		Nitrendipine	ω-Conotoxin GVIA	ω-Agatoxin IVA
KCl				
$\Delta[Ca^{2+}]_i$ (nM)	85.8 ± 2.6 (8)	93.9 ± 4.5 (5)	78.0 ± 7.0 (5)*	57.1 ± 2.4 (4)*
[^{3}H]-DA (%)	4.5 ± 0.4 (9)	—	—	—
Glutamate				
$\Delta[Ca^{2+}]_i$ (nM)	27.7 ± 1.2 (15)	94.2 ± 5.9 (4)	91.7 ± 7.1 (5)	72.5 ± 3.9 (4)*
[^{3}H]-DA (%)	2.1 ± 0.2 (19)	—	—	—
AMPA				
$\Delta[Ca^{2+}]_i$ (nM)	22.4 ± 1.1 (17)	105.2 ± 5.3 (4)	64.8 ± 9.0 (5)*	77.5 ± 4.1 (4)*
[^{3}H]-DA (%)	2.1 ± 0.1 (23)	100.4 ± 5.2 (5)	49.0 ± 3.8 (7)*	46.1 ± 10.5 (5)*
NMDA				
$\Delta[Ca^{2+}]_i$ (nM)	9.1 ± 0.4 (4)	—	—	—
[^{3}H]-DA (%)	3.6 ± 0.2 (13)	91.8 ± 6.5 (6)	94.9 ± 12.1 (5)	98.5 ± 9.0 (5)

NOTE: Experiments were performed as described in FIGURE 1. The Ca^{2+} channel blockers nitrendipine (1 μM), ω-CgTx (0.5 μM), or ω-Aga IVA (100 nM) were added 3 min before stimulation with various agents (5 mM KCl; 100 μM glutamate or AMPA; 200 μM NMDA). Data are presented as mean value ± SEM of changes in $[Ca^{2+}]_i$ (nM) or in evoked [^{3}H]-DA release (%). The effect of various Ca^{2+} channel blockers is expressed as % of the respective control. Asterisk indicates significantly different from control, $p < 0.05$.

[a]Data adapted partially from Malva *et al.*[3,4]

depolarization due to AMPA stimulation triggers the opening of N and P or Q type VSCCs in hippocampal synaptosomes, but not of L type, because nitrendipine had no effect on either $[Ca^{2+}]_i$ or [^{3}H]-DA release induced by AMPA (TABLE 1). The results in TABLE 1 also show that the same types of VSCCs were activated by KCl depolarization as by AMPA stimulation, although the contribution of N type VSCCs is greater with AMPA stimulation than with KCl depolarization.

In the case of glutamate (100 μM) stimulation, we observed that in addition to the effects of glutamate mediated through its receptors, part of the effects on both $[Ca^{2+}]_i$ and [^{3}H]-DA release were due to the interaction of glutamate with its carrier, because the effect could be mimicked by D-aspartate or by t-PDC,[3] a competitive inhibitor of the glutamate carrier. Glutamate transport is electrogenic and depolarizes the membrane of synaptosomes, and VSCCs are also triggered allowing Ca^{2+} entry, which could partially be blocked by ω-Aga IVA, suggesting the involvement of P or Q type VSCCs (TABLE 1).

We conclude that hippocampal synaptosomes are endowed with presynaptic glutamate receptors which modulate the release of dopamine by allowing influx of Ca^{2+} which triggers exocytosis of [^{3}H]-DA. Both AMPA and glutamate induce activation of VSCCs, whereas NMDA appears to modulate [^{3}H]-DA release due to Ca^{2+} entry probably through its receptor channel.

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